

1. A method for the assembly of large DNA fragments, comprising:

- a) manipulating a replicon to comprise in order a final excision site, a first fragment, a first excision site and a first recombinase site;
- b) manipulating a first vector to comprise in order the first recombinase site from step a),
5 undesired vector sequences, the first excision site from step a), a second fragment, and a second excision site;
- c) inserting the first vector into the replicon using a first recombinase so that the two first site specific excision sites are oriented in an appropriate orientation for excision with undesired vector sequences therebetween;
- d) treating the replicon with a first excisionase to remove the undesired vector sequences
10 and bring the second fragment adjacent to the first fragment;
- e) manipulating a second vector to comprise in order the first recombinase site from step a), undesired vector sequences, the second excision site from step b), a third fragment, and the first excision site from step a);
- f) inserting the second vector into the replicon using the first recombinase so that the two
15 second site specific excision sites are oriented in an appropriate orientation for excision with undesired vector sequences therebetween;
- g) treating the replicon with a second excisionase to remove the undesired vector sequences and bring the third fragment adjacent to the second fragment;
- h) repeating steps b-g using at least the first and second excisionases to make an assembled
20 DNA, wherein the final vector also comprises the final excision site 5' to all other sequences and in an appropriate orientation for excision; and
- i) excising and circularizing the assembled DNA with a final excisionase.

2. The method of claim 1, which is performed *in vivo*.

3. The method of claim 1, which is performed *in vitro*.

4. The method of claim 1, wherein the final excisionase site is lox and the final excisionase is cre, the first recombinase is FRT, the first excisionase site is hix and the first excisionase is hin, the second excisionase site is TNE (L/R) and the second excisionase is Tn excisionase.

5 5. The method of claim 1, wherein the assembled DNA fragment (exclusive of the original replicon) is greater than 100, 150, 200, 250, 500, 1000, 1500, 2000, 3000, or 5000 kb.

6. A method for the assembly of large DNA fragments *in vivo*, comprising:

a) manipulating a replicon to comprise in order a first fragment, a first excision site and a first recombinase site;

10 b) manipulating a first vector to comprise in order the first recombinase site from step a), undesired vector sequences, the first excision site from step a), a second fragment, and a second excision site;

15 c) inserting the first vector into the replicon *in vivo* using a first recombinase so that the two first site specific excision sites are oriented in an appropriate orientation for excision with undesired vector sequences therebetween;

d) treating the replicon with a first excisionase *in vivo* to remove the undesired vector sequences and bring the second fragment adjacent to the first fragment;

20 e) manipulating a second vector to comprise in order the first recombinase site from step a), undesired vector sequences, the second excision site from step b), a third fragment, and the first excision site from step a);

f) inserting the second vector into the replicon *in vivo* using the first recombinase so that the two second site specific excision sites are oriented in an appropriate orientation for excision with undesired vector sequences therebetween;

- g) treating the replicon with a second excisionase *in vivo* to remove the undesired vector sequences and bring the third fragment adjacent to the second fragment; and
- h) optionally repeating steps b-g using at least the first and second excisionases *in vivo* to make an assembled DNA.

- 5 7. The method of claim 6, wherein the first recombinase is FRT, the first excisionase site is hix and the first excisionase is hin, the second excisionase site is TNE (L/R) and the second excisionase is Tn excisionase.
8. The method of claim 6, wherein the assembled DNA fragment (exclusive of the original replicon) is greater than 100, 150, 200, 250, 500, 1000, 1500, 2000, 3000, or 5000 kb.